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(71) Applicant *(for all designated States except US)*: GENEMEDICINE, INC. [US/US]; 8301 New Trails Drive, The Woodlands, TX 77381-4248 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROLLAND, Alain [FR/US]; 1719 Prairie Mark, Houston, TX 77077 (US). MUMPER, Russell, J. [US/US]; 19 Heather Wisp Place, The Woodlands, TX 77381 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

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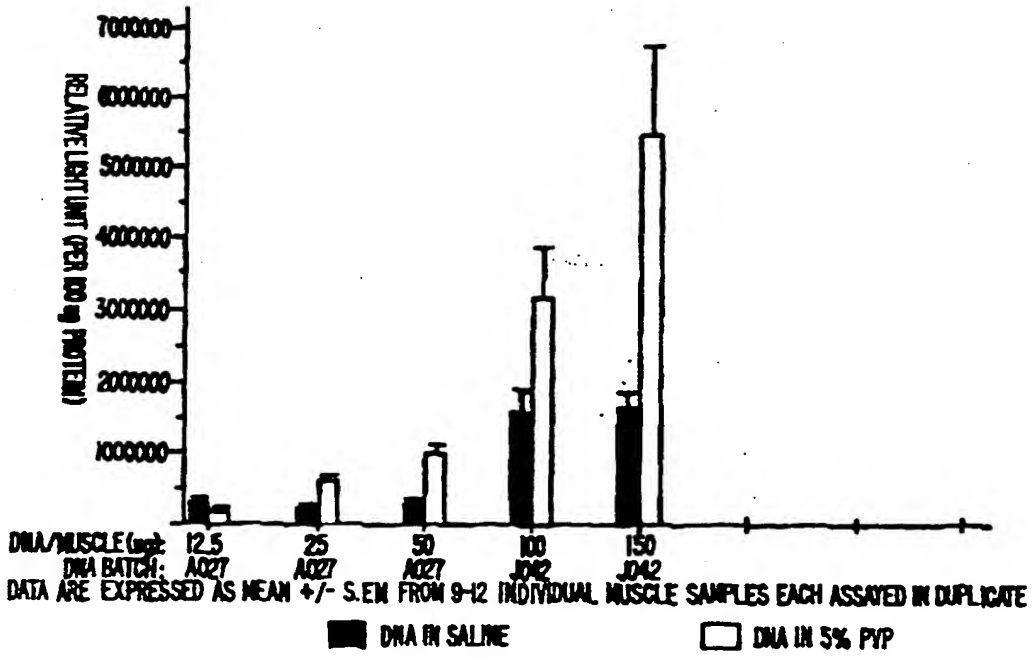

(57) Abstract

Compositions and methods for administering nucleic acid compositions *in vitro* to cells in culture or *in vivo* t an organism whereby the uptake of nucleic acids is enhanced are provided. Various compositions, including thermo-reversible gels, are utilized to increase the viscosity of an administered nucleic acid formulation, thereby prolonging the localized bioavailability f the administered nucleic acid.

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DESCRIPTION

COMPOSITIONS OF NUCLEIC ACID AND VISCOSITY-INCREASING POLYMERS FOR USE IN GENE THERAPY

Background of the Invention

This invention relates to compositions and methods for the introduction of a formulated nucleic acid into a cell for the expression of a peptide or polypeptide. It is useful for *in vitro* transfections and *in vivo* for gene therapy, for among other things administration of therapeutic proteins, polypeptides and peptides and for vaccination.

Non-viral administration of nucleic acid *in vivo* has been accomplished by a variety of methods. These include lipofectin/liposome fusion: Proc. Natl. Acad. Sci., Volume 84, pp. 7413-7417 (1993); polylysine condensation with and without adenovirus enhancement: Human Gene Therapy, Volume 3, pp. 147-154 (1992); and transferrin: transferrin receptor delivery of nucleic acid to cells: Proc. Natl. Acad. Sci., Volume 87, pp. 3410-3414 (1990). The use of a specific composition consisting of polyacrylic acid has been disclosed in WO 94/24983. Naked DNA has been administered as disclosed in WO 90/11092.

An important goal of gene therapy, as an initial step in the process of ultimately obtaining expression of a product encoded by a nucleic acid, is to effect the uptake of nucleic acid by cells. Uptake of nucleic acid by cells is dependent on a number of factors, one of which is the length of time during which a nucleic acid is in proximity to a cellular surface. For instance, after intramuscular (i.m.) administration of plasmid DNA in buffer, a marked reduction in gene expression is observed if the muscle is massaged, presumably due to DNA leakage out of the muscle either directly or via lymphatic vessels (Human Gene Therapy 4:151-159; 1993). Accordingly, it would be desirable to formulate nucleic acids with compounds which

would retard the rate at which nucleic acids diffuse or are carried away from a site at which cellular uptake of the nucleic acid is desired. Further, these compounds would be suitable for administration to an organism by means such as injection while maintaining or regaining the physical characteristics necessary to increase cellular uptake of nucleic acids.

Summary of the Invention

This invention features compositions and methods for enhancing the administration to and uptake of nucleic acids by an organism. An efficient strategy for enhancing nucleic acid delivery in vivo is to maintain the administered nucleic acid at the target site in order to further increase its cellular uptake. Also, for in vitro administration increasing the effective concentration of the nucleic acid at the cell surface should increase the efficiency of transfection. The compositions of the present invention which are used to administer nucleic a acid comprise a compound which prolongs the localized bioavailability of the nucleic acid when administered to an organism or in vitro in cell culture.

By "prolong the localized bioavailability of a nucleic acid" is meant that a nucleic acid when administered to an organism in a composition comprising such a compound will be available for uptake by cells for a longer period of time than if administered in a composition without such a compound, for example when administered in a less viscous formulation such as a saline solution. This increased availability of nucleic acid to cells could occur, for example, due to increased duration of contact between the composition containing the nucleic acid and a cell or due to protection of the nucleic acid from attack by nucleases. The compounds which prolong the localized bioavailability of a nucleic acid are suitable for internal administration.

By "suitable for internal administration" is meant that the compounds are suitable to be administered within the tissue of an organism, for example within a muscle or within a joint space, intradermally or subcutaneously. Other forms of administration which may be utilized are topical, oral, pulmonary, nasal and mucosal; for example, buccal, vaginal or rectal.

By "nucleic acid" is meant both RNA and DNA including: cDNA, genomic DNA, plasmid DNA or condensed nucleic acid, nucleic acid formulated with cationic lipids, nucleic acid formulated with peptides, cationic polymers, RNA or mRNA. In a preferred embodiment, the nucleic acid administered is plasmid DNA which comprises a "vector".

A "vector" is a nucleic acid molecule incorporating sequences encoding therapeutic product(s) as well as, various regulatory elements for transcription, translation, transcript stability, replication, and other functions as are known in the art. A "transcript stabilizer" is a sequence within the vector which contributes to prolonging the half life (slowing the elimination) of a transcript. "Post-translational processing" means modifications made to the expressed gene product. These may include addition of side chains such as carbohydrates, lipids, inorganic or organic compounds, the cleavage of targeting signals or propeptide elements, as well as the positioning of the gene product in a particular compartment of the cell such as the mitochondria, nucleus, or membranes. The vector may comprise one or more genes in a linear or circularized configuration. The vector may also comprise a plasmid backbone or other elements involved in the production, manufacture, or analysis of a gene product. An "expression vector" is a vector which allows for production of a product encoded for by a nucleic acid sequence contained in the vector. For example, expression of a particular growth factor protein encoded by a particular gene. A "DNA vector" is a vector

whose native form is a DNA molecule. A "viral vector" is a vector whose native form is as the genomic material of a viral particle. A "gene product" means products encoded by the vector. Examples of gene products include mRNA templates for translation, ribozymes, antisense RNA, proteins, glycoproteins, lipoproteins and phosphoproteins. The nucleic acid may be associated with a targeting ligand to effect targeted delivery. A "targeting ligand" is a component of the carrier or vehicle which binds to receptors, with an affinity for the ligand, on the surface or within compartments of a cell for the purpose of enhancing uptake or intracellular trafficking of the vector. Tris-galactosyl residues, carnitine derivatives, mannose-6-phosphate, monoclonal antibodies, peptide ligands, and DNA-binding proteins represent examples of targeting ligands which can be used to enhance uptake. "Targeted delivery" involves the use of targeting ligands which specifically enhance translocation of a nucleic acid to specific tissues or cells. A "target" is a specific organ, tissue, or cell for which uptake of a vector and expression of a gene product is intended. "Uptake" means the translocation of the vector from the extracellular to intracellular compartments. This can involve receptor mediated processes, fusion with cell membranes, endocytosis, potocytosis, pinocytosis or other translocation mechanisms. The vector may be taken up by itself or as part of a complex. "Binding" is an intermediate step in uptake of some complexes involving a high-affinity interaction between a targeting ligand and a surface receptor on a target cell. "Intracellular trafficking" is the translocation of the vector within the cell from the point of uptake to the nucleus where expression of a gene product takes place. Alternatively, cytoplasmic expression of a nucleic acid construct utilizing, for example, a T7 polymerase system may be accomplished. Various steps in intracellular trafficking include endosomal release and compartmentalization of the vector within various extranuclear compartments,

and nuclear entry. "Endosomal release" is the egress of the vector from the endosome after endocytosis. This is an essential and potentially rate limiting step in the trafficking of vectors to the nucleus. A lytic peptide may be used to assist in this process. A "lytic peptide" is a peptide which functions alone or in conjunction with another compound to penetrate the membrane of a cellular compartment, particularly a lysosomal or endosomal compartment, to allow the escape of the contents of that compartment to another cellular compartment such as the cytosolic and/or nuclear compartment. "Compartmentalization" is the partitioning of vectors in different compartments within a defined extracellular or intracellular space. Significant extracellular compartments may include, for example, the vascular space, hair follicles, interstitial fluid, synovial fluid, cerebral spinal fluid, thyroid follicular fluid. Significant intracellular compartments may include endosome, potosome, lysosome, secondary lysosome, cytoplasmic granule, mitochondria, and the nucleus. "Nuclear entry" is the translocation of the vector across the nuclear membrane into the nucleus where the gene may be transcribed. "Elimination" is the removal or clearance of materials (vectors, transcripts, gene products) from a specific compartment over time. This term may be used to reflect elimination from the body, the vascular compartment, extracellular compartments, or intracellular compartments. Elimination includes translocation (excretion) from a particular compartment or biotransformation (degradation).

The compounds which prolong the localized bioavailability of a nucleic acid may also achieve one or more of the following effects, due to their physical, chemical or rheological properties: (1) Protect nucleic acid, for example plasmid DNA, from nucleases due to viscosity effects; (2) increase the area of contact between nucleic acid, such as plasmid DNA, through extracellular matrices and over cellular membranes, into which the nucleic acid

is to be taken up; (3) concentrate nucleic acid, such as plasmid DNA, at cell surfaces due to water exclusion; (4) indirectly facilitate uptake of nucleic acid, such as plasmid DNA, by disrupting cellular membranes due to osmotic, hydrophobic or lytic effects. The following polymers, oils and surfactants may be suitable for use as compounds which prolong the localized bioavailability of a nucleic acid: Celluloses, including salts of carboxymethylcelluloses, methylcelluloses, hydroxypropyl-celluloses, hydroxypropylmethylcelluloses; salts of hyaluronates; salts of alginates; heteropolysaccharides (pectins); poloxamers (Pluronics); poloxamines (Tetronics); ethylene vinyl acetates; polyethylene glycols; dextrans; polyvinylpyrrolidones; chitosans; polyvinylalcohols; propylene glycols; polyvinylacetates; phosphatidylcholines (lecithins); miglyols; polylactic acid; polyhydroxybutyric acid. These substances may be prepared as solutions, suspensions, gels, emulsions or microemulsions of a water/oil (w/o), water/oil/water (w/o/w) or oil/water (o/w) or oil/water/oil (o/w/o) type. Oil suspensions of lyophilized nucleic acid, such as plasmid DNA may be utilized. Carriers for these oil suspensions include, but are not limited to, sesame oil, cottonseed oil, soybean oil, lecithins, Tweens, Spans and Miglyols. By "solutions" is meant water soluble polymers and/or surfactants in solution with nucleic acids. By "suspensions" is meant water insoluble oils containing suspended nucleic acids. By "gels" is meant high viscosity polymers containing nucleic acids. By "emulsion" is meant a dispersed system containing at least two immiscible liquid phases. Emulsions usually have dispersed particles in the 0.1 to 100 micron range. They are typically opaque and thermodynamically unstable. Nucleic acids in the water phase can be dispersed in oil to make a w/o emulsion. This w/o emulsion can be dispersed in a separate aqueous phase to yield a w/o/w emulsion. Alternatively, a suitable oil could be dispersed in an aqueous

phase to form an o/w emulsion. A "microemulsion" has properties intermediate to micelles and emulsions and is characterized in that they are homogenous, transparent and thermodynamically stable. They form spontaneously when oil, water, surfactant and cosurfactant are mixed together. Typically, the diameter of the dispersed phase is 0.01 to 0.1 microns, usually of the w/o and o/w type.

The compounds which prolong the bioavailability of a nucleic acid may also interact or associate with the nucleic acid by intermolecular forces and/or valence bonds such as: Van der Waals forces, ion-dipole interactions, ion-induced dipole interactions, hydrogen bonds, or ionic bonds. These interactions may serve the following functions: (1) Stereoselectively protect nucleic acids from nucleases by shielding; (2) facilitate the cellular uptake of nucleic acid by "piggyback endocytosis". Piggyback endocytosis is the cellular uptake of a drug or other molecule complexed to a carrier that may be taken up by endocytosis. CV Uglea and C Dumitriu-Medvichi. Medical Applications of Synthetic Oligomers. In: Polymeric Biomaterials. Edited by Severian Dumitriu. Marcel Dekker, Inc. 1993, incorporated herein by reference. To achieve the desired effects set forth it is desirable, but not necessary, that the compounds which prolong the bioavailability of a nucleic acid have amphipathic properties; that is, have both hydrophilic and hydrophobic regions. The hydrophilic region of the compounds may associate with the largely ionic and hydrophilic regions of the nucleic acid, while the hydrophobic region of the compounds may act to retard diffusion of nucleic acid and to protect nucleic acid from nucleases. Additionally, the hydrophobic region may specifically interact with cell membranes, possibly facilitating endocytosis of the compound and thereby nucleic acid associated with the compound. This process may increase the pericellular concentration of nucleic acid. Agents which may have amphipathic properties and

are generally regarded as being pharmaceutically acceptable are the following: Methylcelluloses, hydroxypropylcelluloses, hydroxypropylmethylcelluloses; heteropolysaccharides (pectins); poloxamers (Pluronics); poloxamines (Tetronics); ethylene vinyl acetates; polyethylene glycols; polyvinylpyrrolidones; chitosans; polyvinylalcohols; polyvinylacetates; phosphatidylcholines (lecithins); propylene glycol; miglyols; polylactic acid; polyhydroxybutyric acid; xanthan gum. Also, copolymer systems such as polyethylene glycol-polylactic acid (PEG-PLA), polyethylene glycol-polyhydroxybutyric acid (PEG-PHB), polyvinylpyrrolidone-polyvinylalcohol (PVP-PVA), and derivatized copolymers such as copolymers of N-vinyl purine (or pyrimidine) derivatives and N-vinylpyrrolidone.

In one embodiment of the invention, the compound which prolongs the bioavailability of a nucleic acid is a sustained-release compound which may be administered to an organism or to cells in culture. The sustained-release compound containing a nucleic acid is administered to the tissue of an organism, for example, by injection. In one embodiment the tissue is preferably muscle tissue. In another embodiment the tissue is preferably a joint space. By "sustained-release compound" is meant a substance with a viscosity above that of an isotonic saline solution (150 mM NaCl) containing a nucleic acid; for example, DNA in saline at 1 mg/ml has a viscosity of 3.01 mPa·sec, DNA in saline at 2 mg/ml has a viscosity of 3.26 mPa·sec, DNA in saline at 3 mg/ml has a viscosity of 5.85 mPa·sec (Viscosity measurements were performed at 25°C in a Brookfield DV-III Rheometer with a No. 40 Spindle at 75 rpm for 30 minutes). Preferably the sustained-release compound has a viscosity in the range of about 0.1-20,000 mPa·sec above that of a formulation in which isotonic saline is the carrier for a nucleic acid. More preferably the range is about 0.1-5000 mPa·sec above that of a formulation in which isotonic saline is the carrier for a nucleic acid. Even more preferably the range is about

0.1-1000 mPa·sec above that of a formulation in which isotonic saline is the carrier for a nucleic acid. By "sustained-release" is meant that nucleic acid is made available for uptake by surrounding tissue or cells in culture for a period of time longer than would be achieved by administration of the nucleic acid in a less viscous medium, for example, a saline solution.

In another embodiment, the compound which prolongs the bioavailability of a nucleic acid is a thermo-reversible gel. By "thermo-reversible gel" is meant a gel which is substantially liquid at temperatures below about 30°C but forms a gel at temperatures above about 30°C. Administration of the thermo-reversible gel by, for example, injection is thereby facilitated if the gel is cooled so that it is in a substantially liquid state when injected. However, upon contact with the tissue of an organism which is at a temperature of above about 30°C the viscosity of the thermo-reversible gel increases, thereby increasing the localized bioavailability of a nucleic acid formulated with the thermo-reversible gel.

In another embodiment of the present invention, the molecules of the compound which prolongs the localized bioavailability of a nucleic acid tend to orient themselves in the direction of an induced flow and as an applied force causing the flow is increased and the resistance of the compound to flow is decreased, lowering an initial viscosity of the compound. When the applied force is removed, the compound substantially reverts to its initial viscosity. In a preferred embodiment the compound utilized is a salt of carboxymethylcellulose, such as sodium carboxymethylcellulose. Sodium carboxymethylcellulose has been used by the cosmetics, food, and pharmaceutical industries as a stabilizer, thickener, gelling agent, suspending agent, and a lubricant. Sodium carboxymethyl cellulose is an approved pharmaceutical excipient.

In another embodiment, the compound which prolongs the bioavailability of a nucleic acid is polyvinylpyrrolidone (PVP). PVP is a polyamide that forms complexes with a wide variety of substances and is chemically and physiologically inert. Specific examples of suitable PVP's are Plasdone-C®15, MW 10,000 and Plasdone-C®30, MW 50,000.

In another embodiment the compound which prolongs the bioavailability of a nucleic acid is an oily suspension. By "oily suspension" is meant a coarse dispersion containing finely divided insoluble material suspended in a liquid medium. These formulations include: nucleic acids, polymers, peptides or sugars and are dispersed with the aid of a dispersing agent, such as a surfactant in a suitable vehicle such as an oil. For example, DNA/PVP powder blend in Miglyol with 0.1% Tween-80, DNA/PVP powder blend in sesame oil with 0.1% Tween-80, DNA/lactose powder blend in Miglyol with 0.1% Tween-80, DNA complex powder blends in Miglyol with 0.1% Tween-80, where the DNA complex could comprise condensed DNA complexes such as DNA:polymer or DNA:peptide.

In another embodiment the compound which prolongs the bioavailability of a nucleic acid is a water-in-oil microemulsion. Examples would include: lecithin:sesame oil:butanol (surfactant/oil/cosurfactant) as the oil phase with DNA in saline as the water phase; lecithin:sesame oil:butanol (surfactant/oil/cosurfactant) as the oil phase with DNA complex saline as the water phase.

In another embodiment the compound which prolongs the bioavailability of a nucleic acid is a hydrogel. Nucleic acids may be loaded into hydrogels by placing swellable hydrogel systems in nucleic acid solutions. Swellable hydrogels include but are not limited to hydroxyethylmethacrylate (HEMA), polyethyleneglycolmethacrylate (PEGMA), cellulose ether hydrogels, comprising cross-linked hydroxypropyl cellulose, methyl cellulose, and hydroxypropylmethyl

cellulose; calcium-crosslinked alginate; crosslinked polyvinyl alcohols and Poloxamers (Pluronics).

In another embodiment the compound which prolongs the bioavailability of a nucleic acid is a cationic polymer, such as Eudragit, Chitosan and Poloxamines (Tetronics).

In another embodiment the compound which prolongs the bioavailability of a nucleic acid is a surfactant which forms micelles, such as Tween 80.

In another embodiment the uptake of nucleic acids in vitro, for example, cells in tissue culture is enhanced by the use of the compounds disclosed herein.

Other and further objects, features, and advantages will be apparent from the following description of the presently preferred embodiments in the invention.

Brief Description of the Drawings

Figure 1 illustrates a plot of the fraction of PVP:DNA at different ratios remaining within a dialysis sac over time.

Figure 2 illustrates the transfection efficiency into C_2C_{12} myoblasts of a plasmid DNA complex administered with various polymers and controls of polymer alone, and pDNA complex alone.

Figure 3 illustrates the transfection efficiency into C_2C_{12} myoblasts when transfected with plasmid DNA in 10%, 20%, and 30% PEG (8 kDa) with and without the presence of an endosomal release peptide (lytic peptide).

Figure 4 illustrates the magnitude of β-galactosidase marker gene expression when a plasmid containing the marker gene is administered in saline or a PVP formulation.

Figure 5 illustrates the time course of β-galactosidase expression in PVP as compared to the time course in saline.

Figure 6 illustrates that the biologically excretable low molecular weight PVP is equally effective as the high molecular weight species in transfecting muscle tissue.

Figure 7 is an illustration of the results of a gel-electrophoresis undertaken to quantitate the degree of DNA degraded by DNAse I in saline versus PVP.

Figure 8 is an illustration of the effect of administering DNA containing a CAT reporter gene in 5% mannitol versus saline.

Figure 9 is an illustration of the effect of administering PVA containing a CMV-β-galactosidase reporter gene in high molecular weight (40,000 daltons) at 1% PVA or 1.7% PVA and low molecular weight (18,000 daltons) at 2% PVA or 4% PVA.

Figure 10 shows the CMV-Beta-Glactosidase Expression in Tibialis Muscle. The graph shows a comparison of various gene delivery systems. Relative light units per 100 ug of protein are shown for (1) mock; (2) DNA in saline; and (3) DNA in 10.8% propylene glycol.

The drawings are not necessarily to scale. Certain features of the invention may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness.

Detailed Description of the Invention

Sodium carboxymethylcellulose is a long chain cellulose ether polymer. Many types are commercially available varying as to molecular weight (degree of polymerization) and percent carboxymethyl esterification per 10 cellulose units (degree of substitution). When mixed with water the polymers form viscous solutions which possess unique rheological characteristics. Polymerized types of cellulose ethers exhibit pseudoplastic and thixotropic behavior. By thixotropic behavior is meant that the long-chain molecules tend to orient themselves in the direction of flow; as the applied force is increased, the resistance to flow is decreased. Yet when high shear stress is removed, the solution will quickly revert to its original viscous state. Some celluloses exhibit thixotropic behavior wherein the solution returns to its

viscous state over a period of time. The pseudoplasticity and thixotropic properties of sodium carboxymethylcellulose can be utilized for intramuscular injection of nucleic acid, such as plasmid DNA. A formulation of the viscous solution of sodium carboxymethylcellulose in isotonic saline containing plasmid DNA becomes fluid due to the pressure of injection by a syringe and needle then thicken once deposited in the muscle. The thickening of the injected formulation in situ provides retention of the expression vector within the muscle resulting in a controlled and sustained release and an enhanced uptake of the vector by the muscle cells.

In an alternative embodiment a thermoreversible gel may be used. After i.m. administration, plasmid DNA is maintained within the muscle by using a thermo-reversible gel formulation. The use of compounds that are aqueous at ambient temperature, yet are gels at body temperatures (e.g. 37°C for humans) are used to ease the formulation and administration of the DNA yet transition to and maintain the gel state for increased bio-availability at temperatures encountered in vivo. Such formulations (thermo-reversible gels) are prepared by adjusting the concentrations of polymers in aqueous solutions so that the vector delivery system will be liquid at room temperature or below and will be in the form of a gel in situ in the muscle at physiologic temperatures. Poloxamers (Pluronic F127®, Poloxamer 407®), poloxamines and the concentration of the polymers may be adjusted according to the formulation depending upon the route of administration (i.e., topical, i.m.,) for nucleic acid or nucleic acid complexes. These adjustments may be found in United States Patent 5,292,516 which is incorporated by reference herein.

The following examples are offered by way of illustration and are not intended to limit the scope of the invention in any manner.

Example 1

Demonstration of PVP Plasmid DNA Complex Formation

A given amount of lyophilized plasmid DNA is rehydrated with water and made isotonic with sterile 5M NaCl. After complete rehydration, an appropriate volume of sterile stock PVP solution in water is added to result in the desired final PVP concentration in isotonic saline. Alternatively, if plasmid DNA is already in solution, the appropriate volumes of plasmid DNA, 5M NaCl and stock PVP solutions are added to result in the desired proportions. The complex is allowed to form at 25°C after gentle shaking. For example:

Ingredient	Amount
lyophilized DNA	1 mg
sterile water	0.770 ml
25% PVP in water	0.2 ml
5M NaCl	0.030 ml
Final formulation:	1 mg DNA/1 ml of 5% PVP in isotonic saline.

A dynamic dialysis experiment with three complexes was undertaken to determine the retention of PVP (MW = 10 kDa) within dialysis sacs. Spectra/Por CE (cellulose ester) membranes with a MW cut-off of 25 kDa were employed. Three 1 ml formulations and corresponding controls were placed in pre-washed sacs, the sacs were closed and suspended in 100 ml saline at 25°C. The formulations and controls were as follows:

Formulations	PVP/DNA (w/w)
90 mg PVP and 0.5 mg CMV-β-gal in saline	180:1
60 mg PVP and 0.5 mg CMV-β-gal in saline	120:1
30 mg PVP and 0.5 mg CMV-β-gal in saline	60:1
Controls	**PVP/DNA (w/w)**
90 mg PVP and 0.5 mg CMV-β-gal in saline	180:0
60 mg PVP and 0.5 mg CMV-β-gal in saline	120:0
30 mg PVP and 0.5 mg CMV-β-gal in saline	60:0
0.5 mg CMV-β-gal in saline	0:1

Aliquots were taken over 24 hours. The results are shown in Figure 1. The fraction of PVP remaining in the dialysis sac was plotted over time. In all cases, the rate of PVP diffusion through the dialysis membrane was decreased in the presence of plasmid DNA, indicating complex formation between PVP and plasmid DNA at the three weight ratios tested. It was also determined that the sac volume remained constant during the duration of the experiment and that adherence of PVP to the membrane was negligible.

Example 2

Demonstration of Increased Transfection Efficiency With Amphipathic Polymers

It was demonstrated that amphipathic polymers such as PVP (MW = 50 kDa), PEG (MW = 8 kDa), and Poloxamer 407 can increase the efficiency of cell transfection, in-vitro in studies carried out using C_2C_{12} myoblasts. Transfections were made using combinations of a complex and various polymers. The plasmid DNA complex consisted of plasmid DNA:condensing agent:Peptide 1 (1:64:3 -/+/-) (The condensing agent may be those as are known in the art, for example, dendrimers or polylysine). (Peptide-1 is a lytic peptide. A peptide similar to Peptide 1 is described in United States Patent application S.N. 07/913,669, filed July 14, 1992). The results show that at higher concentrations, 7.5% of the amphipathic polymers: PVP; PEG; and Poloxamer, the transfection efficiency of the plasmid DNA complex was significantly enhanced over the plasmid DNA complex alone or the polymers alone. Conversely, with the largely ionic polymer, CMC, the synergistic effect was not observed. This may have been due to destabilization of the net positively charged plasmid DNA complex by the negatively charged CMC. While not being limited in scope by any theory set forth, several mechanisms of action of amphipathic polymers may account for the observed results including: Stabilization

of plasmid DNA complexes due to coating; increased cell membrane permeability, thereby allowing easier passage of the plasmid DNA complex through the cell; membrane and/or volume exclusion, increasing the concentration of plasmid DNA complexes at the cell surface. Poloxamer 407 has been shown to improve the transduction efficiency of adenoviral vectors by apparently maintaining a high pericellular concentration of the vector or by disrupting the cell membrane. K. March et al. Facilitation of Adenoviral Gene Delivery by Poloxamer 407. Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 21 (1994).

Example 3

Demonstration of Increased Transfection Efficiency Utilizing a Lytic Peptide

C_2C_{12} myoblasts were transfected with 4 um plasmid DNA in 10%, 20%, and 30% PEG (8 kDa) with and without the presence of 6 ug of an endosomal release peptide (lytic peptide) The results are shown in Figure 3. With no lytic peptide, only at 20% PEG as a carrier did transfection result. 10% and 30% PEG carriers did not give transfection. Additionally, when the lytic peptide was included, the transfection efficiency was enhanced 100-fold for the 20% PEG carrier. This result suggested the importance of a lytic agent in the carrier system, but also that the plasmid DNA in 20% PEG (without the peptide) was probably being taken up by the cell but degraded in the lysosomes. It has been found that 20% PEG is optimal for transfecting Micromonospora with bacteriophage DNA. JL Caso et al. Transfection in Micromonospora. Appl. Environ. Microbiol. 1987; 53 (10): 2544-47. The ability of the 20% PEG carrier to transfect cells is attributed to its ability to interact with plasmid DNA. Hydrodynamic light scattering data has shown that 20% PEG but not 10% PEG or 30% PEG can collapse plasmid DNA, presumably due to water exclusion.

Example 4

Demonstration of Enhanced Nucleic Acid Uptake and Expression Utilizing PVP

Polyvinylpyrrolidone (PVP) is a polyamide that forms complexes with a wide variety of substances and is chemically and physiologically inert. Applicants have shown that PVP enhances nucleic acid uptake/expression in muscle and should prove useful in delivering nucleic acids for the prophylactic treatment of diseases.

A CMV-β-galactosidase expression vector system was formulated in saline or 5% PVP and administered into the tibialis muscle of a rat. The activity of β-galactosidase gene product was measured in muscle extract at various time intervals after injection.

Delivery of DNA-PVP Formulation into Muscle: 5-6 week old male rats (Fisher 344 strain, 120-130 g) from Harlan Sprague-Dawley laboratories were used. The animals were housed in microisolators at Baylor Animal Facility and maintained on a 12 h/12 h day/night cycle, with room temperature at 72° F, and at 40% humidity. Animals were anesthetized with a mixture of Ketamine (42.8 mg/ml), Xylazine (8.6 mg/ml) and Acepromazine (1.4 mg/ml) at a dose of 0.5 - 0.7 ml/kg, i.m. A 2-4 mm incision was made aseptically and 50 μl of a DNA formulation in PVP or saline was injected into the tibialis muscle of both legs. At various time intervals after injection, animals were anaesthetized, sacrificed by thoracotomy and the tibialis muscle was harvested and collected on dry ice and stored at -70°C until assayed for β-galactosidase activity.

Extraction and Measurement of β-Galactosidase Activity in Muscle Injected with DNA-PVP Formulation:

β-galactosidase was extracted with 1.5 ml of Tris-EDTA-NaCl buffer containing the protease inhibitors leupeptin (1 μM), pepstatin (1 μM) and PMSF (0.25 mM). The extract was centrifuged at 13 K rpm for 15 min at 4°C.

The supernatant was collected and 100 μg protein was assayed for β-galactosidase activity using a chemiluminescence detection system. An example of a suitable system is the Galacto-Light™ or Galacto-Light Plus™ available from Tropix, Inc. of Bedford, Massachusetts. Galacto-Light™ and Galacto-Light Plus™ are a chemiluminescent reporter assay systems designed for the rapid, sensitive, and non-isotropic detection of β-galactosidase in cell lysates. The Galacto-Light™ (Galacto-Light Plus™) reporter assay incorporates Galacton™ (Galacton-Plus™) chemiluminescent substrate for β-galactosidase with Emerald™ luminescence enhancer. The chemiluminescent assay has a wide dynamic range, enabling detection of 2 fg to 20 ng of β-galactosidase. Jain, V., and I. Magrath. A Chemiluminescent Assay for Quantitation of β-Galactosidase in the Femtogram Range: Application to Quantitation of β-Galactosidase in lacZ-Transfected Cells. Anal. Biochem. 199: 119-124 (1991) incorporated herein by reference. Galacton™ chemiluminescent substrate has a half-life of light emission of approximately 4.5 minutes after the addition of Galacto-Light™ accelerator. It is suited for use with luminometers with automatic injectors and other instrumentation in which light emission measurements can be taken within a short period of time. Luminometer measurements taken within a narrow time frame make results more accurate and simple to interpret. Galacton-Plus™ chemiluminescent substrate emits light which persists at a constant level for up to 60 minutes after the addition of Galacto-Light™ accelerator. This substrate is ideal for use with either plate luminometers that do not have automatic injection capabilities or with scintillation counters. Cell lysate or purified β-galactosidase is incubated with reaction buffer for 15 minutes to 1 hour. Galacton™ (Galacton-Plus™) chemiluminescent substrate present in the reaction buffer is cleaved by the enzyme. The sample is then placed in a luminometer chamber and a light emission accelerator is

added which terminates the β-galactosidase activity and accelerates light emission. Light output is quantitatively measured using a 5 second integral. It is important to stay within the linear range of the assay, especially if β-galactosidase is being used to normalize transfections. High signals can potentially saturate a photo-multiplier tube resulting in artificially low signals. In addition, low signals that approach background levels may also be outside the linear range. In these cases, the amount of cell extract used in the assay should be adjusted to bring the assay within the linear range. The Galacto-Light™ (Galacto-Light Plus™) system has been formulated for luminometers with a 300μl automatic injector. When using Galacto-Light™, manual injection may be performed if luminescence intensities are measured at approximately the same interval after adding the light emission accelerator to each sample. However, Galacto-Light Plus™ eliminates this need due to the long half-life of light emission exhibited by Galacton-Plus™. Reaction components should be scaled down if a luminometer with a smaller volume injector is used, however, sensitivity may be affected slightly. For plate luminometers it will be necessary to scale down the reaction volumes proportionately. However, it is recommended to keep the volume of cell extract between 5 and 20μl. The Lysis solution included with the kit may be substituted with alternative lysis solutions and lysis procedures. This may be desirable if assays for other co-transfected reporters require specific assay buffers. Alternative lysis solutions should be compared with the Galacto-Light™ Lysis Solution to ensure optimal performance of the assay. Chemiluminescent reporter assays may be conducted in cells or tissues that have endogenous β-galactosidase activity. Endogenous enzyme activity is slightly reduced at the pH of the Galacto-Light™ Reaction Buffer, while bacterial β-galactosidase encoded on transfected plasmids is only slightly affected.

In this case, it is important to assay the level of endogenous enzyme with non-transfected cell extracts. Significant reductions of endogenous activity can be achieved using heat inactivation. Tissue extracts may also require the use of protease inhibitors. The following reagents are used: Chemiluminescent Substrate: Galacton™ or Galacton-Plus™ is a 100X concentrate which is diluted in reaction buffer diluent prior to use (store at 4°C or optimally at -20°C); Lysis Solution containing 100 mM potassium phosphate pH 7.8, 0.2% Triton X-100 (Store at 4°C). Dithiothrietol (DTT) should be added fresh prior to use to a final concentration of 1mM; Reaction Buffer Diluent containing 100 mM sodium phosphate pH 8.0, 1 mM magnesium chloride (store at 4°C); Accelerator contains a ready-to-use luminescence accelerator reagent (store at 4°C).

Preparation of Cell Extracts From Tissue Culture Cells

(1) Aliquot the required amount of Lysis Solution. Add fresh DTT to 1 mM. (2) Rinse cells 2 times with 1X Phosphate Buffered Saline (PBS). (3) Add Lysis Solution to cover the cells (250µl of Lysis Buffer for a 60 mm culture plate should be adequate). (4) Detach cells from culture plate using a rubber policeman or equivalent. Non-adherent cells should be pelleted and lysis buffer should be added sufficient to cover the cells. The cells should then be resuspended in the lysis buffer by pipetting. (5) Transfer cells to a microfuge tube and centrifuge for 2 minutes to pellet any debris. (6) Transfer supernatant to a fresh microfuge tube. Cell extracts may be used immediately or frozen at -70°C for future use.

Chemiluminescent Detection Procedure

It is recommended that all assays are performed in triplicate. (1) Dilute Galacton™ (Galacton-Plus™) substrate 100-fold with Galacto-Light™ Reaction Buffer

Diluent to make Reaction Buffer. This mixture will remain stable for several months if stored uncontaminated at 4°C. It is recommended to only dilute the amount of substrate that will be used within a two month period. (2) Warm to room temperature the amount of Reaction Buffer required for the entire experiment. (3) Aliquot 2 to 20µl of individual cell extracts into luminometer sample tubes. (The amount of cell extract required may vary depending on the amount of expression and the instrumentation used. Use 5µl of extract for positive controls and 10 to 20 µl of extract for experiments with potentially low levels of enzyme. It is important to vary the concentrations of extract to keep the signal within the linear range of the assay.) (4) Add 200 µl of Reaction Buffer to a luminometer cuvette and gently mix. Incubate at room temperature for 60 minutes. Incubations can be as short as 15 minutes, but the linear range of the assay may decrease. (Measurements are time dependent. Reaction Buffer should be added to sample extracts in the same time frame as they are counted on the luminometer. For example, if it takes 10 seconds to completely count a sample, then Reaction Buffer should be added to tubes every 10 seconds.) (5) Place cuvette in a luminometer. Inject 300µl of Accelerator. After a 2 to 5 second delay following injection, count the sample for 5 seconds. If manual injection is used, then the Accelerator should be added in the same consistent time frame as the Reaction Buffer is added. This is critical when using Galacton™.

Preparation of Controls

Positive Control

Add 1 µl of β-galactosidase (10 units/ml, Sigma Cat. No. G-5635 diluted in 0.1 M sodium phosphate pH 7.0, 1.0% BSA) to mock transfected cell extract equivalent to the volume of experimental cell extract used. Proceed with Chemiluminescent Detection Procedure.

Negative Control

Assay of volume of mock transfected cell extract equivalent to the volume of experimental cell extract used. Proceed with Chemiluminescent Detection Procedure.

Heat Inactivation of Endogenous β-galactosidase

Some cell lines may exhibit relatively high levels of endogenous β-galactosidase activity. This may lead to background which will decrease the overall sensitivity of the assay by lowering the signal to noise ratio. A procedure for heat inactivation of endogenous β-galactosidase activity has been described by Young *et al.* Young, Dorothy C., S.D. Kingsley, K.A. Ryan, and F.J. Dutko. Selective Inactivation of Eukaryotic β-Galactosidase in Assays for Inhibitors of HIV-1 TAT Using Bacterial β-Galactosidase as a Reporter Enzyme. Anal. Biochem. 215:24-30 (1993), incorporated herein by reference. A modified version of this protocol has also been described by Shaper *et al.* in which a cocktail of protease inhibitors is used in conjunction with the heat inactivation procedure for reducing β-galactosidase in tissue extracts. Shaper, N., Harduin-Lepers, A., and Shaper, H.H. Male Germ Cell Expression of Murine β4-Galactosyltransferase. A 796-base pair genomic region containing two cAMP-responsive elements (CRE)-like elements, mediates expression in transgenic mice. J. Biol. Chem. 269:25165-25171 (1994), incorporated herein by reference.

Inactivation of β-Galactosidase Activity in Cell Extracts

The following procedures should be performed immediately prior to the Chemiluminescent Detection Procedure in the Preparation of Cell Extracts From Tissue Culture Section. (1) Following cell extract preparation, heat the extract to 48°C for 50 minutes. (2) Proceed with Chemiluminescent Detection Procedure. (Although Young *et*

al. suggest 50°C for 60 minutes, heat inactivation at 48°C for 50 minutes is suggested.)

Inactivation of Endogenous β-Galactosidase Activity in Tissue Extracts

(1) To the Galacto-Light™ lysis buffer, add PMSF to a final concentration of 0.2 mM and leupeptin to a final concentration of 5 μg/ml just before use. (2) Heat inactivate the extracts by heating at 48°C for 60 minutes. (3) Proceed with Chemiluminescent Detection Procedure. (AEBSF (Sigma Cat. No. A-5938) may be used in place of PMSF (Sigma Cat. No. P-7626). AEBSF is a water soluble serine protease inhibitor).

A liquid scintillation counter may be used as a substitute for a luminometer, however, sensitivity may be lower Fulton, R., and B. Van Ness. Luminescent Reporter Gene Assays for Luciferase and β-galactosidase Using a Liquid Scintillation Counter. BioTechniques 14(5): 762-763(1993), incorporated herein by reference. Nguyen, V.T., M. Morange, and O. Bensaude. Firefly Luciferase Luminescence Assays Using Scintillation Counters for Quantitation in Transfected Mammalian Cells. Anal. Biochem. 171:404-408 (1988), incorporated herein by reference. The results are expressed as mean +/- S.E.M of Relative Light Unit, as indicative of b-galactosidase activity, per 100 ug muscle protein. When using a scintillation counter, it is necessary to turn off the coincident circuit in order to measure chemiluminescence directly. The manufacturer of the instrument should be contacted to determine how this is done. If it is not possible to turn off the coincident circuit, a linear relationship can be established by taking the square root of the counts per minute measured and subtracting the instrument background. Actual = (measured-instrument background)$^{1/2}$. Other methods of measuring a chemiluminescent signal as are known in the art may also be utilized.

Results: Intramuscular administration of CMV-β-galactosidase expression vector formulated in either saline or 5% PVP (International Specialty Products, Plasdone-C®15, m.w. 10,000 and Plasdone-C®30, m.w. 50,000, Pharmaceutical grade) resulted in the expression of β-galactosidase enzyme in the transfected muscles. The magnitude and time course of β-galactosidase expression was compared between the saline and PVP formulations. As shown in Figure 4, the magnitude of expression was considerably higher when the DNA was formulated in PVP (50,000 MW) as compared to saline. The enhancement of β-galactosidase expression by PVP over saline was dependent on the dose of DNA injected. At a low DNA dose (12.5 ug/injection) there was no difference in the expression level between PVP and saline formulations. At a higher DNA dose (25-150 ug) the level of expression in PVP was higher compared to saline formulation. The DNA dose response in saline formulation reached a plateau at 25 ug whereas it continued to increase in a linear fashion in PVP formulation in the dose range studied. To further characterize the DNA-PVP formulation, the time course of β-galactosidase expression in PVP was compared with the time course in saline. As shown in Figure 5, the difference between PVP and saline formulations was maintained throughout the time course. Maximum difference was observed at day 15 after injection. Experiments were also conducted with low molecular weight PVP (10,000 daltons). As shown in Figure 6, the biologically excretable low molecular weight PVP is equally effective as the high molecular weight species in transfecting muscle tissue. The high and low molecular weight PVP was administered at different concentrations, resulting in solutions with the same viscosity.

Example 5

Physical Studies on PVP and PVP-DNA Interactions

PVP-DNA interactions by FTIR: Fourier-Transformed Infra Red (FTIR) has been used to investigate PVP-DNA interactions. From this study, it has been shown that PVP stabilizes the backbone of the DNA, as indicated by the sharpness of bands 970 cm^{-1} and 1086 cm^{-1}. The FTIR also suggests a decrease in the resonance character of the bases. There is also a broadening and decrease in intensity from 1400-1600 cm-1 and increased resolution and intensity from 1650-1800 cm^{-1} and 1200-1400 cm^{-1}. This is interpreted as a decrease in resonance character associated with the DNA bases when PVP is present. The result is the formation of explicit double and single bond formation. The FTIR (>1650 cm-1) also suggests a greater distinction in the environment between exocyclic base residues due to splits in the degeneracy when the PVP is present. Alternatively, the apparent splits may result from the presence of the carbonyl stretching mode associated with PVP.

Mechanism of Action: While the invention is not to be limited by any particular theory, as mechanisms of action, it is postulated that PVP may act as follows:

1. It may protect the DNA by altering the diffusion of nucleases within the polymer matrix. It may also provide water exclusion which may reduce nuclease activity. Consistent with a physical interaction between DNA and PVP, isothermal titration calorimetry measurements using a Hart Scientific microtitration calorimeter gave a positive heat of binding. These measurements indicate a positive enthalpy and suggest that PVP:DNA interactions are driven by the displacement of water or counterions.
2. PVP, through its hydrophobic regions may be capable of interacting, even fusing with biological membranes.
3. When PVP is at the surface of the cell, it will concentrate the associated DNA at that surface. If PVP is

also fusogenic, it could then transfer the localized DNA into the cytoplasm.

Example 6

Protection of DNA from DNAse I Degradation

Protection of DNA in formulation compositions from DNAse I degradation by 5% PVP was demonstrated. Solutions of DNA alone or DNA in 5% PVP (50 kDa) were prepared at 37°C in saline DNAse I Activity Buffer (50 mM sodium acetate, pH 6.5 with 10 mM $MgCl_2$, 2 mM $CaCl_2$). The concentration of DNA was 100 ug/ml of Activity Buffer. To all solutions, various amounts of DNAse I in Activity Buffer were added. The weight ratios of DNAse I to DNA were: 1:250,000, 1:50,000, 1:25,000, 1:12,000. The manufacturer of the DNAse I used indicates that 1:10,000 DNAse I to DNA will entirely degrade DNA at 37°C in 15 minutes. The samples were allowed to incubate at 37°C for 15 minutes, at which time an aliquot of each sample was added to tracking dye. As shown in Figure 7, gel-electrophoresis was undertaken to quantitate the degree of degraded DNA. The results showed that higher amounts of DNAse I were needed to degrade DNA in the presence of PVP as compared to the absence of PVP.

Example 7

Increase in Expression of a Reporter Gene Administered in Mannitol Versus Saline

As shown in Figure 8, the effect of administering DNA containing a chloramphenicol acetyltransferase (CAT) reporter gene in 5% mannitol versus saline was investigated. The use of CAT as a reporter gene is well known in the art. A typical protocol may be found in Current Protocols in Molecular Biology, Chapter 9, Unit 9.6A Reporter System Using Chloramphenicol Acetyltransferase © 1993 Current Protocols. As shown in Figure 8, the expression of the CAT reporter gene in 5% mannitol was approximately four times that when

administered in saline. The DNA was administered to the tibalis muscle of rats as described above in Example 4.

Example 8

Increase in Expression of a Reporter Gene Administered in PVA Versus Saline

As shown in Figure 9, the effect of administering DNA containing a CMV-β-galactosidase reporter gene in high molecular weight PVA (40,000 daltons) at 1% PVA or 1.7% PVA and low molecular weight PVA (18,000 daltons) at 2% PVA or 4% PVA was investigated. The low molecular weight PVA at both 2% and 4% gave approximately two and six times expression, respectively, of the reporter gene as saline. Determination of the level of expression of the reporter gene was performed as described above in Example 4. The DNA was administered to the tibalis muscle of rats as described above in Example 4.

Example 9

Increase in Expression of a Reporter Gene Administered in PEG Versus Saline

As shown in Figure 10, the effect of administering DNA in 10.8% propylene glycol versus saline was investigated. As shown in that Figure, the expression of the CMV-β-galactosidase reporter gene in 10.8% propylene glycol was approximately three times that when administered in saline. Determination of the level of expression of the reporter gene was performed as described above in Example 4. The DNA was administered to the tibalis muscle of rats as described above in Example 4.

It will be readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

Claims

1. A composition comprising a mixture of a compound, which prolongs the localized bioavailability of a nucleic acid, and a nucleic acid, said composition being suitable for internal administration to an organism.

2. The composition of claim 1, wherein said compound which prolongs the localized bioavailability of a nucleic acid comprises a sustained-release compound.

3. The composition of claim 1, wherein the molecules of said compound which prolongs the localized bioavailability of a nucleic acid tend to orient themselves in the direction of an induced flow and wherein as an applied force causing the flow is increased the resistance of said composition to flow is decreased, lowering an initial viscosity of said composition, further wherein when said applied force is removed said composition substantially reverts to said initial viscosity.

4. The composition of claim 1, wherein said nucleic acid is selected from the group consisting of condensed nucleic acid, nucleic acid formulated with cationic lipids, nucleic acid formulated with peptides, or cationic polymers.

5. The composition of claim 2, wherein said nucleic acid is selected from the group consisting of condensed nucleic acid, nucleic acid formulated with cationic lipids, nucleic acid formulated with peptides, or cationic polymers.

6. The composition of claim 3, wherein said nucleic acid is selected from the group consisting of condensed nucleic acid, nucleic acid formulated with cationic

lipids, nucleic acid formulated with peptides or cationic polymers.

7. The composition of claim 2, wherein said sustained-release compound comprises a salt of carboxymethylcellulose.

8. The composition of claim 7, wherein said salt of carboxymethylcellulose comprises sodium carboxymethylcellulose.

9. The composition of claim 2, wherein said sustained-release compound comprises polyvinylpyrrolidone.

10. The composition of claim 2, wherein said sustained-release compound comprises polyethyleneglycol.

11. The composition of claim 1, wherein said compound which prolongs the localized bioavailability of a nucleic acid has an initial viscosity in the range of 0.1-20,000 mPa·sec greater than that of a solution in which isotonic saline is a carrier.

12. The composition of claim 7, wherein said salt of carboxymethylcellulose has an initial viscosity in the range of 0.1-20,000 mPa·sec.

13. The composition of claim 8, wherein said sodium carboxymethylcellulose has an initial viscosity in the range of 0.1-20,000 mPa·sec.

14. The composition of claim 9, wherein said polyvinylpyrrolidone has an initial viscosity in the range of 0.1-20,000 mPa·sec.

15. The composition of claim 10, wherein said polyethyleneglycol has an initial viscosity in the range of 0.1-20,000 mPa·sec.

16. A composition comprising a sustained-release compound and a nucleic acid suitable for use in vitro.

17. The composition of claim 16, wherein said nucleic acid is selected from the group consisting of condensed nucleic acid, nucleic acid formulated with cationic lipids, nucleic acid formulated with peptides or cationic polymers.

18. The composition of claim 16, wherein said sustained-release compound comprises a salt of carboxymethylcellulose.

19. The composition of claim 18, wherein said salt of carboxymethylcellulose is sodium carboxymethyl cellulose.

20. The composition of claim 16, wherein said sustained-release compound comprises polyvinylpyrrolidone.

21. The composition of claim 16, wherein said sustained-release compound comprises polyethyleneglycol.

22. A composition comprising a mixture of a thermo-reversible gel and a nucleic acid, said composition suitable for internal administration to an organism.

23. The composition of claim 22, wherein said thermo-reversible gel is liquid at temperatures below about 30°C but forms a gel at temperatures above about 30°C.

24. The composition of claim 22, wherein said nucleic acid is selected from the group consisting of condensed nucleic acid, nucleic acid formulated with cationic lipids, nucleic acid formulated with peptides or cationic polymers.

25. The composition of claim 22, wherein said thermo-reversible gel comprises a poloxamer.

26. The composition of claim 22, wherein said poloxamer comprises Pluronic F127®.

27. The composition of claim 22, wherein said thermo-reversible gel comprises a poloxamine.

28. A composition comprising a thermo-reversible gel and a nucleic acid suitable for use in vitro.

29. The composition of claim 28, wherein said nucleic acid is selected from the group consisting of condensed nucleic acid, nucleic acid formulated with cationic lipids, nucleic acid formulated with peptides or cationic polymers.

30. The composition of claim 28, wherein said thermo-reversible gel comprises a poloxamer.

31. The composition of claim 28, wherein said poloxamer comprises Pluronic F127®.

32. The composition of claim 28, wherein said thermo-reversible gel comprises a poloxamine.

33. A method of administering a composition comprising a compound which prolongs the localized bioavailability of a nucleic acid and a nucleic acid

comprising the step of introducing said composition into the muscle of an organism.

34. The method of claim 33, wherein said step of introducing said composition into the muscle of an organism is by injection.

35. A method of administering a composition comprising a compound which prolongs the localized bioavailability of a nucleic acid and a nucleic acid comprising the step of introducing said composition into the interstitial joint space of an organism.

36. The method of claim 35, wherein said step of introducing said composition into the interstitial joint space of an organism is by injection.

FIG. 1.

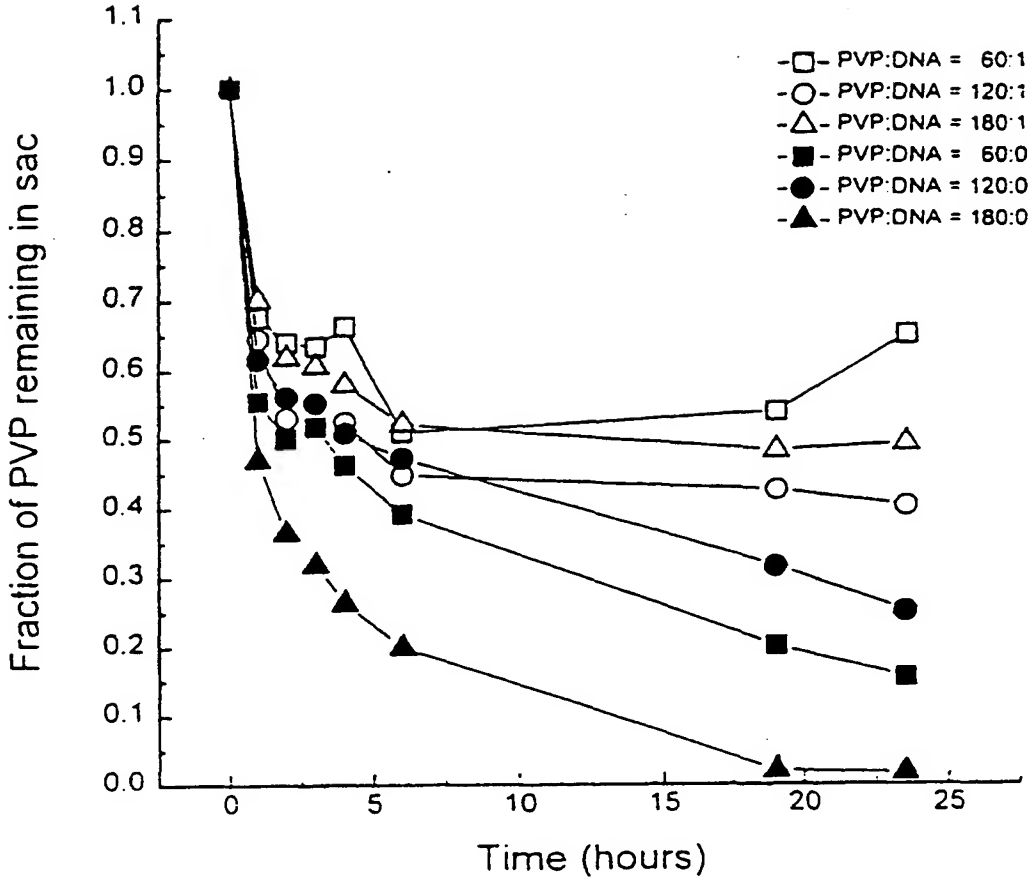

Fraction of PVP remaining in sac
Time (hours)
-□- PVP:DNA = 60:1
-○- PVP:DNA = 120:1
-△- PVP:DNA = 180:1
-■- PVP:DNA = 60:0
-●- PVP:DNA = 120:0
-▲- PVP:DNA = 180:0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
0
5
10
15
20
25

FIG. 2.

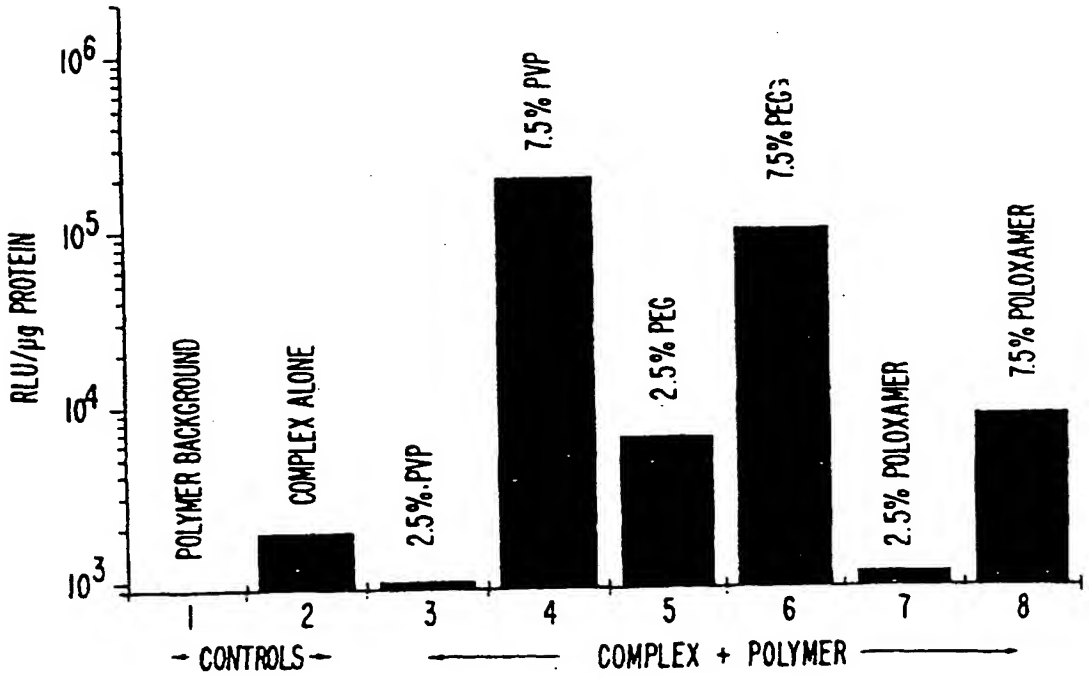

RLU/µg PROTEIN
10^3
10^4
10^5
10^6
POLYMER BACKGROUND
COMPLEX ALONE
2.5% PVP
7.5% PVP
2.5% PEG
7.5% PEG
2.5% POLOXAMER
7.5% POLOXAMER
1
2
3
4
5
6
7
8
CONTROLS
COMPLEX + POLYMER

FIG. 3.

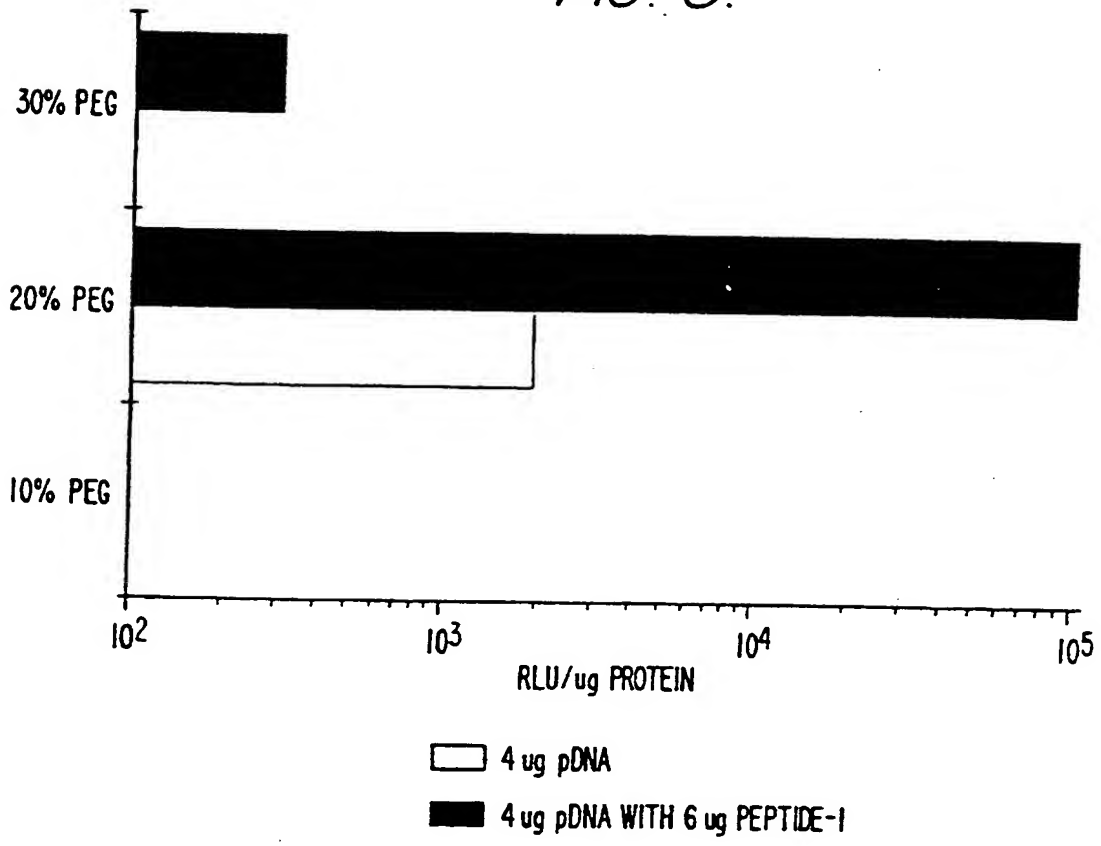

30% PEG
20% PEG
10% PEG
10^2
10^3
10^4
10^5
RLU/ug PROTEIN
4 ug pDNA
4 ug pDNA WITH 6 ug PEPTIDE-1

FIG. 4.

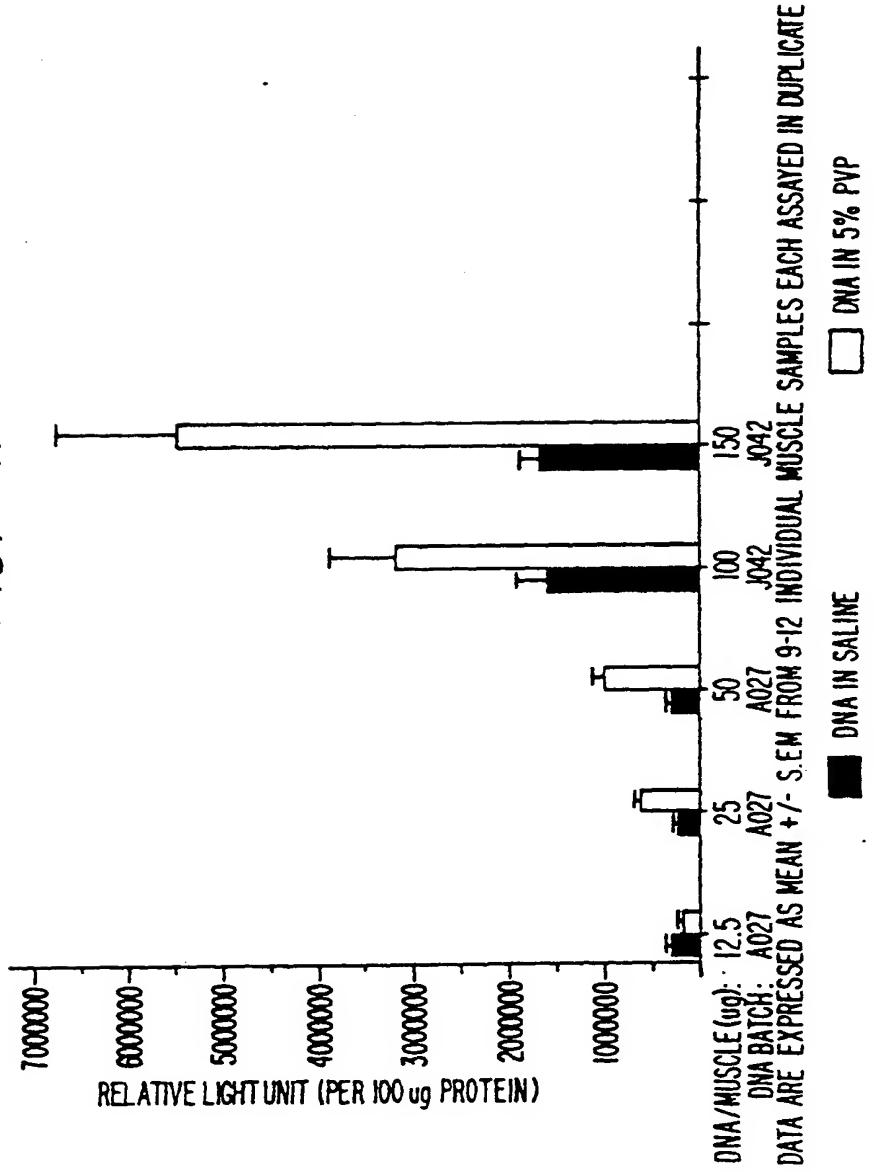
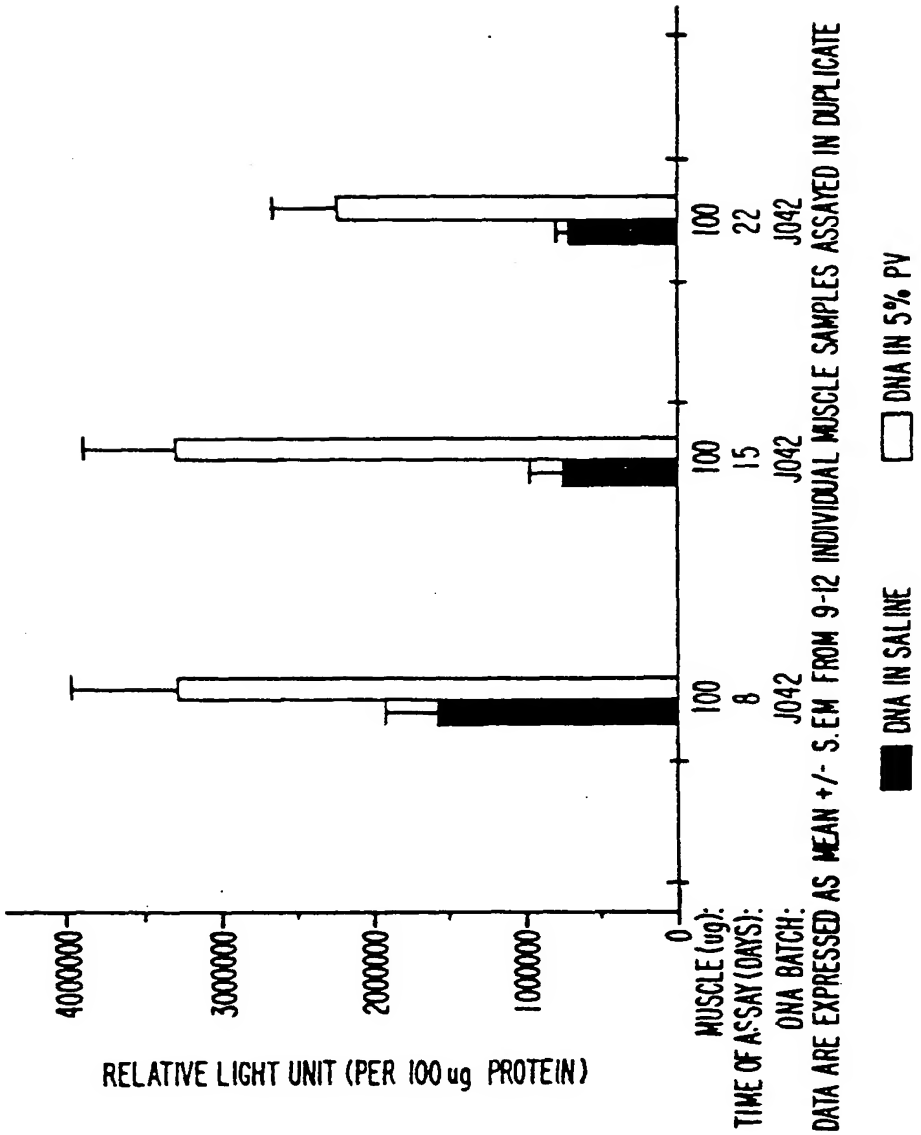

RELATIVE LIGHT UNIT (PER 100 ug PROTEIN)
7000000
6000000
5000000
4000000
3000000
2000000
1000000
DNA/MUSCLE (ug): 12.5 25 50 100 150
DNA BATCH: A027 A027 A027 J042 J042
DATA ARE EXPRESSED AS MEAN +/- S.E.M FROM 9-12 INDIVIDUAL MUSCLE SAMPLES EACH ASSAYED IN DUPLICATE
DNA IN SALINE
DNA IN 5% PVP

FIG. 5.


4000000
3000000
2000000
1000000
0
RELATIVE LIGHT UNIT (PER 100 ug PROTEIN)
MUSCLE (ug):
TIME OF ASSAY (DAYS):
DNA BATCH:
100
8
J042
100
15
J042
100
22
J042
DATA ARE EXPRESSED AS MEAN +/- S.E.M FROM 9-12 INDIVIDUAL MUSCLE SAMPLES ASSAYED IN DUPLICATE
DNA IN SALINE
DNA IN 5% PV

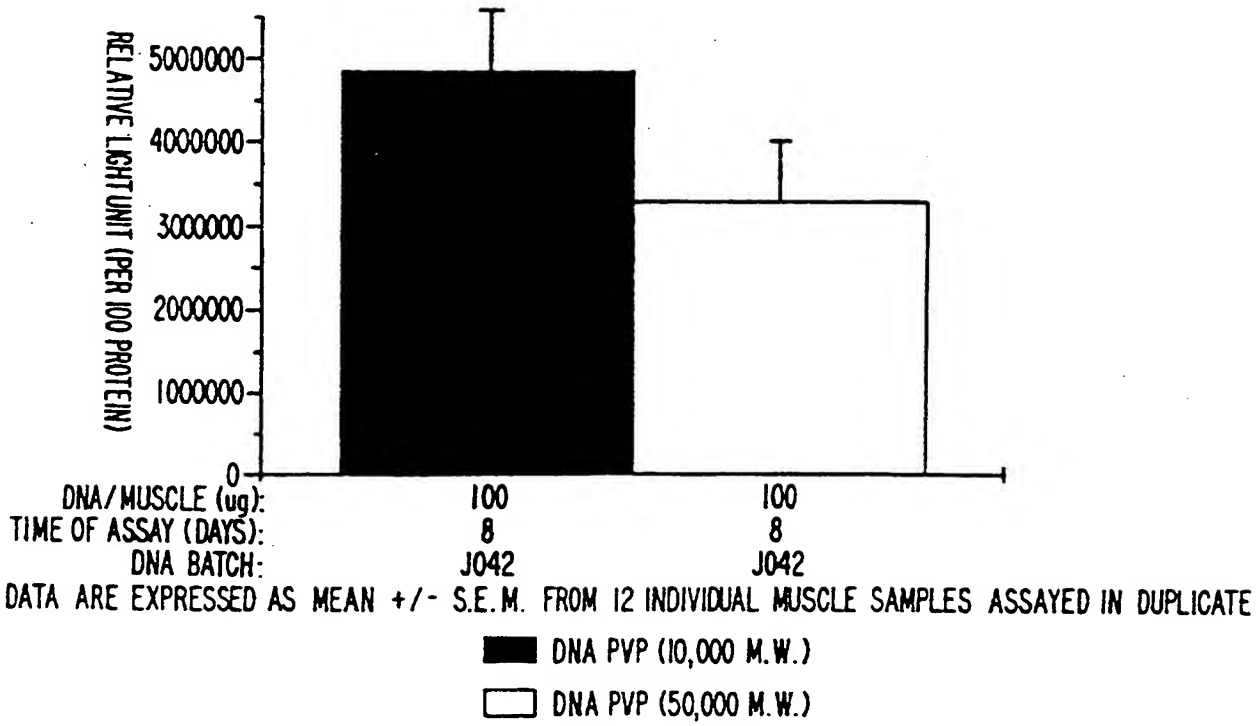

RELATIVE LIGHT UNIT (PER 100 PROTEIN)
5000000
4000000
3000000
2000000
1000000
0
DNA/MUSCLE (ug): 100 100
TIME OF ASSAY (DAYS): 8 8
DNA BATCH: J042 J042
DATA ARE EXPRESSED AS MEAN +/- S.E.M. FROM 12 INDIVIDUAL MUSCLE SAMPLES ASSAYED IN DUPLICATE
DNA PVP (10,000 M.W.)
DNA PVP (50,000 M.W.)

FIG. 6.

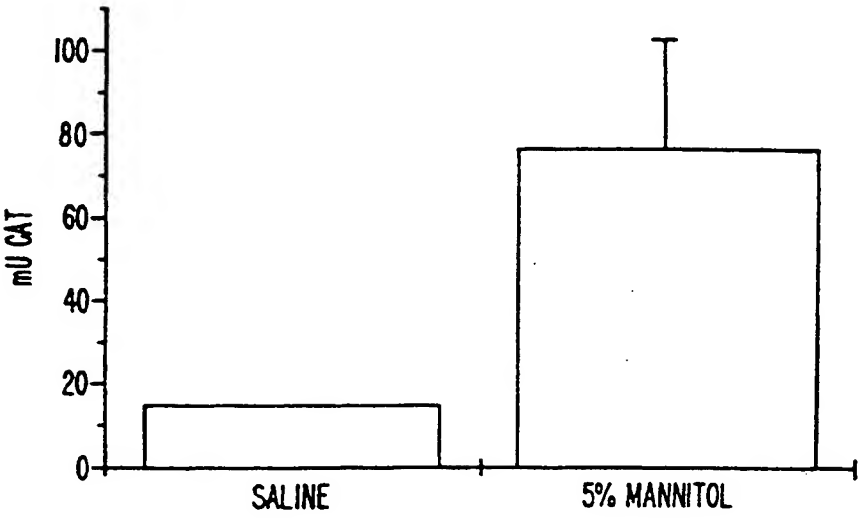

mU CAT
100
80
60
40
20
0
SALINE
5% MANNITOL

FIG. 8.

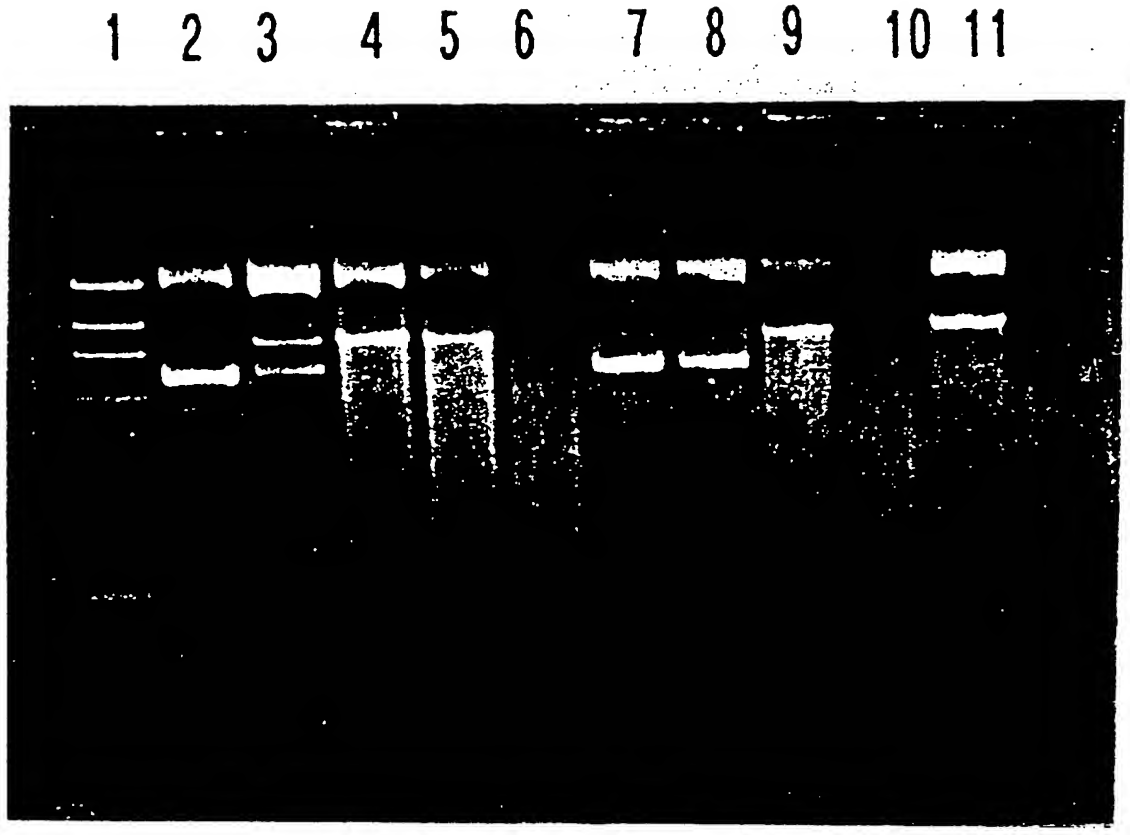


FIG. 7.

FIG. 9.

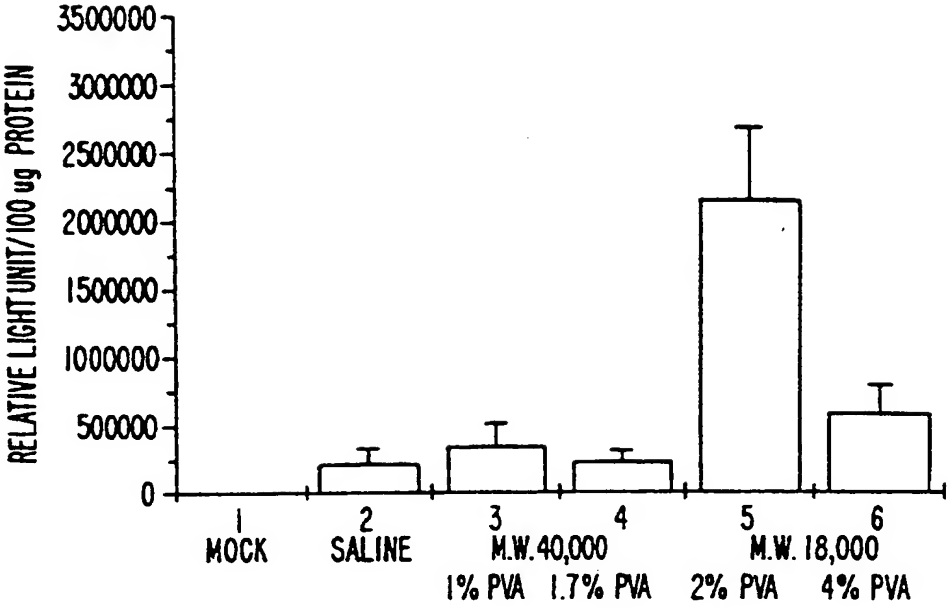
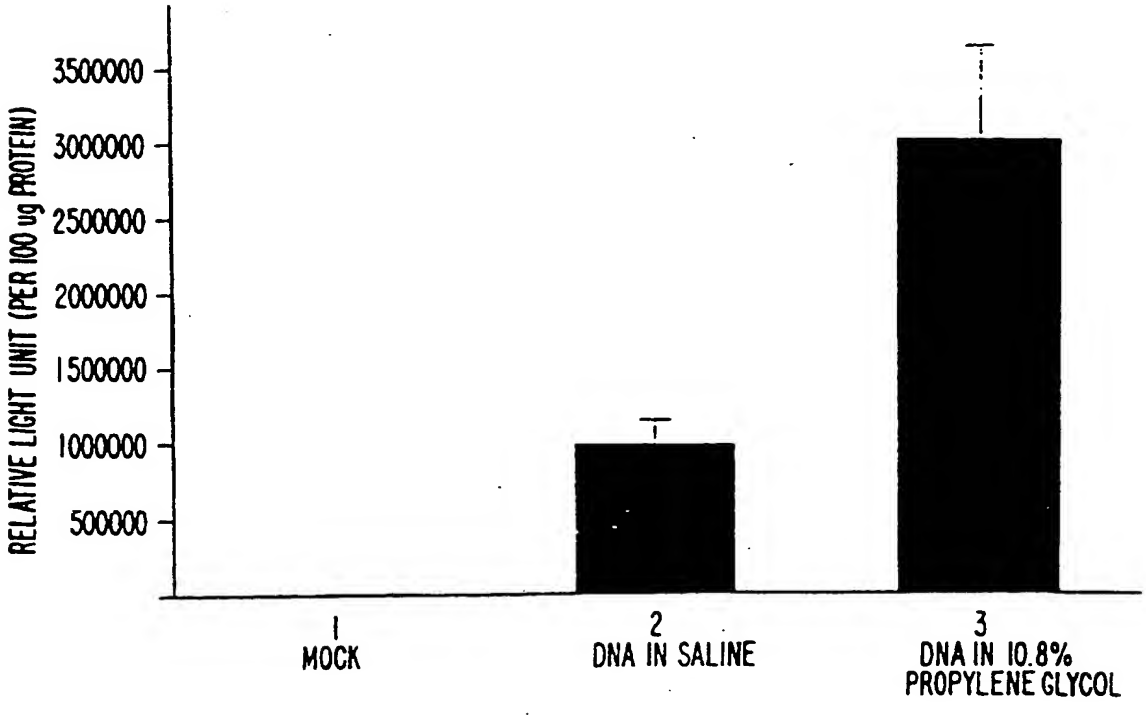

RELATIVE LIGHT UNIT/100 ug PROTEIN
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
1
MOCK
2
SALINE
3
4
M.W.40,000
1% PVA
1.7% PVA
5
6
M.W. 18,000
2% PVA
4% PVA

FIG. 10


RELATIVE LIGHT UNIT (PER 100 ug PROTEIN)
3500000
3000000
2500000
2000000
1500000
1000000
500000
1
MOCK
2
DNA IN SALINE
3
DNA IN 10.8%
PROPYLENE GLYCOL

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: GENEMEDICINE, INC. [US/US]; 8301 New Trails Drive, The Woodlands, TX 77381-4248 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROLLAND, Alain [FR/US]; 1719 Prairie Mark, Houston, TX 77077 (US). MUMPER, Russell, J. [US/US]; 19 Heather Wisp Place, The Woodlands, TX 77381 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

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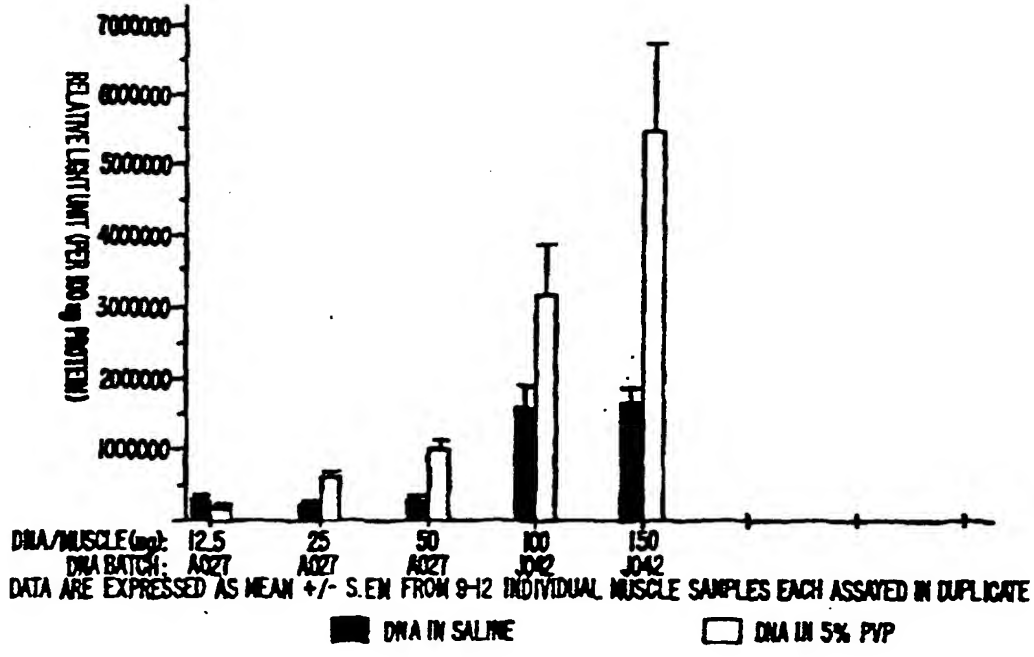
(54) Title: COMPOSITIONS OF NUCLEIC ACID AND VISCOSITY-INCREASING POLYMERS, FOR USE IN GENE THERAPY



(57) Abstract

Compositions and methods for administering nucleic acid compositions *in vitro* to cells in culture or *in vivo* to an organism whereby the uptake of nucleic acids is enhanced are provided. Various compositions, including thermo-reversible gels, are utilized to increase the viscosity of an administered nucleic acid formulation, thereby prolonging the localized bioavailability f the administered nucleic acid.

INTERNATIONAL SEARCH REPORT

International Application No
US 95/17038

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K48/00 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J CLIN INVEST, FEB 1994, VOL. 93, NO. 2, PAGE(S) 820-8, XP000572774 BENNETT MR ET AL: "Inhibition of vascular smooth muscle cell proliferation in vitro and in vivo by c-myc antisense oligodeoxynucleotides." see abstract see page 821, right-hand column ---	1-36
X	TERATOG., CARCINOG., MUTAGEN., 1986, VOL 6, NO. 3, PAGES 245-50, XP000577777 KLEBE, ROBERT J. ET AL: "Uptake by cells of nucleic acids promoted by compounds sharing the pleiotropic effects of poly(ethylene glycol)" see abstract see paragraph: materials and methods --- -/--	1-36

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 1996	16.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Dullaart, A

Form PCT/ISA/210 (second sheet) (July 1992)

Inte onal Application No
/US 95/17038

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PHARMACEUTICAL RESEARCH, SEPT 1995, VOL. 12, NO. 9 SUPPL., PAGE(S) S80., ABSTR. BIOTEC 2005, XP000574000 MUMPER R J ET AL: "Interactive polymeric gene delivery systems for enhanced muscle expression" see the whole document	1-36
X,P	CIRC. RES., FEB. 1995, VOL. 76, NO. 2, PAGE(S) 176-182, XP000572772 EDELMAN E.R. ET AL: "c-myc in vasculoproliferative disease" see abstract see paragraph: Results	1-36
P,Y	ARCHIVES DES MALADIES DU COEUR ET DES VAISSEAUX, MAR. 1995, VOL. 88, NO. 3, PAGE(S) 381-389, FRANCE, XP000572776 CHEMLA E. ET AL: "ACTION DES OLIGONUCLEOTIDES ANTISENS SUR L'HYPERPLASIE MYO-INTIMALE DANS UN MODELE DE TRAUMATISME D'AORTE ABDOMINALE DE RAT" see abstract see paragraph: Méthodes	1-36
Y	J BIOL CHEM, 1980, VOL. 255, NO. 21, PAGE(S) 10431-10435, XP002007683 FRALEY R ET AL: "INTRODUCTION OF LIPOSOME ENCAPSULATED SV-40 DNA INTO CELLS" see abstract see paragraph: materials and methods	1-36
Y	BIOCHEMISTRY, VOL. 9, NO. 22, PAGES 4396-401, 1970, XP002007684 WEITH, HERBERT L. ET AL: "Synthesis of cellulose derivatives containing the dihydroxyboryl group and a study of their capacity to form specific complexes with sugars and nucleic acid components" see abstract see paragraph: Experimental section	1-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/17038

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 33-36 are directed to a method of treatment/diagnosis of the human/animal body, the search has been carried out, based on the alleged effects of the compounds/composition (PCT Rule 39.1(iv)).

2. [X] Claims Nos.: 1-36
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
please see continuation sheet ./.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

MEANINGFUL SEARCH NOT POSSIBLE OR INCOMPLETE SEARCH

2. Obscurities, ..etc.

In view of the large number of compounds, which are defined by the gen ral definitions of the components used in the claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see Guidelines, part B, Chapter III, paragraph 3.6).